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14. ABSTRACT

Prostate cancer results from complex interactions among genetic, endocrine, and environmental factors. Understanding genetic risk factors that contribute to the occurrence of prostate cancer is crucial to design both preventative and therapeutic strategies and to identify at-risk individuals. This knowledge could reduce the incidence of and death from this disease. The primary objective of this grant is to investigate changes in genes that directly and indirectly regulate levels of male hormones, which in turn, affect prostate cell growth, and may ultimately cause cancer. In this past year, we have performed genotyping for variants in INS and IRS2 to analyze in conjunction with the IRS1 and IGF1 variant genotypes to look at main effects and gene x gene interactions. In addition, we investigated haplotypes in *IGFBP1* and *SHBG* in order to assess the association with variation across the entire gene. Statistical methods were used to analyze the association of these genes with occurrence of prostate cancer, age at diagnosis and disease aggressiveness. The *IRS1* variant was associated with a 2.7 fold increased risk of prostate cancer.

15. SUBJECT TERMS
Prostate cancer, genetics, molecular variants, genetic epidemiology

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Annual Progress Report
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INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer in men and the second most common cause of cancer mortality in the United States. One in five men will be diagnosed with prostate cancer over the course of a lifetime. In our aging population, research leading to a reduction in the incidence of and mortality from prostate cancer is an urgent necessity. A critical problem in prostate cancer is an understanding of risk factors involved in disease development and aggressiveness. Clinically important genetic risk factors that may result in differences in individual susceptibility to prostate cancer likely include genes involved in androgen biosynthesis, metabolism and regulation and in prostate cell growth and death. We propose to answer the following questions. What are some of the genetic risk factors that determine who develops prostate cancer? Of those individuals who develop cancer, what risk factors contribute to the age at diagnosis and to aggressiveness of the disease? Using a case-control design, we are testing the hypothesis that common genetic polymorphisms (variants) in genes directly and indirectly involved in altering hormonal levels and prostate cell growth are associated with prostate cancer risk. We are investigating their associations with occurrence of prostate cancer, age at diagnosis, and aggressiveness of the disease as measured by Gleason score and tumor stage-related variables.

BODY

Our progress is described by Tasks. We are studying 199 prostate cancer cases and 254 age-matched controls. Data on family history, age at diagnosis, and clinical and pathological characteristics have been obtained for the prostate cancer cases. We are genotyping for the genetic variants in DNA samples from this set of cases and controls. In the proposal, we had selected a set of variants within genes to investigate. Since that time, we have decided to also more fully screen a subset of these genes and to screen additional genes in the insulin-like growth factor (IGF) signaling pathway, a pathway that regulates both cell growth and death. Researchers have traditionally focused on one or two variants in a gene, so that a negative association has not excluded the gene, but only those specific variants. We have modified the proposal to use powerful new approaches that combine multiple linked variants in a single gene to form haplotypes. For *SHBG, IRS1, IGFPB1*, and *IGFBP3*, we will look at variation across the entire gene as described under task 9. In this past period, we have also decided to look at genetic variation in *IGF1* and *IGF1R*, as they are critical in the IGF pathway.

Aim 1: To assay samples for the genetic variants (genotyping).

Task 1: Design allele-specific primers for genotyping. Test and optimize the genetic assays. Compare with published protocol results. Sequence to confirm that detecting the appropriate alleles. COMPLETED.

We are currently performing single nucleotide polymorphism (SNP) genotyping using the Taqman exonuclease assay. In instances where a Taqman assay cannot be designed, we are designing primers to genotype using the T_m-shift genotyping assay. For design of the Taqman assays, we first use Applied Biosystems (ABI) Assay-by-Design service. We send them sequence encompassing the SNP and they design and optimize the assays. For those that ABI could not design, we send sequence to EPOCH. They also use the Taqman exonuclease assay, but the probe design is different. The majority of SNPs that could not be designed by Assay-by-Design could be designed by EPOCH. We then test the assay when we receive it using known homozygotes and heterozygotes for the variants. For the 41 haplotype-tagging

SNPs in *SHBG, IRS1, IGFBP1, IGFBP3, IGF1,* and *IGF1R*, 31 assays are from ABI Assay by Design and 8 are from EPOCH. We have now tested and optimized the assays and all work well. We confirm the assay results by sequencing a subset. For two of the SNPs, neither Taqman exonuclease assays nor T_m-shift genotyping assays worked. For these two SNPs, we will use restriction endonuclease assays. Thus, during this past period, we identified the SNPs for *IRS1, IGF1,* and *IGF1R*, sent off sequence, and tested and optimized the assays.

Task 2: Screen for variants in insulin-like growth factor binding protein I (*IGFBP-1*) to identify a variant(s) for genotyping. COMPLETED PREVIOUSLY.

We identified 19 variants of which 3 SNPs are required to tag the variation in this gene in Caucasians. These three haplotype-tagging SNPs were used for genotyping in Task 5.

Task 3: Identify male controls which match prostate cancer cases. COMPLETED PREVIOUSLY.

For the 199 prostate cancer cases for whom we have DNA and diagnosis and follow-up data, we have identified 254 age-matched male population-based controls. Dr. Brothman was no longer able to enroll participants in his study, so there are no additional cases or controls available for this study.

Task 4: Aliquot DNA from all samples available. COMPLETED PREVIOUSLY.

However, in this past year, we were running low on 50 of the DNA samples. Therefore, we performed whole genome amplification on those samples using a kit from Amersham in order to increase the amount of DNA. We had previously validated that the DNA after whole genome amplification provided the same results as the non-amplified DNA.

Task 5: Perform genotyping.

The total DNA samples available for genotyping are 453, 199 prostate cancer cases and 254 controls. During previous funding periods, we had completed genotyping on the 199 prostate cancer cases and 254 controls for the following polymorphisms: *CYP17-MspI, IGF-1* STR, *CYP11A* STR, *VDR-BsmI, VDR-Taq1, VDR-polyA, IRS1* G972R, *SHBGI* D327N, *SRD5A2-str*, the *INS* +1127 Ins-PstI and *IRS2* G1079D SNPs. We also performed genotyping for the three haplotype-tagging SNPs in *IGFBP1* and the four in *SHBG*. During this past period, we genotyped the samples for the PI3KCB and the SHC1 variants, genotyped 2 additional SNPs in *IRS1*, genotyped one of the *IGFBP3* SNPs, and 8 of the *IGF1R* SNPs.

In the next year, we will genotype the DNA samples for eight haplotype-tagging SNPs in *IGF1*, an additional ten SNPs in *IGF1R*, one additional SNP in *IRS1* and three additional SNPs in *IGFBP3*, as well as the genotyping of the *SRD5A2* V89L and *CYP3A4* B1 polymorphisms from the original proposal.
Note: In a previous report, we described that we had decided that it was not useful to genotype the microsatellite repeat markers in *HSD3B2* and *HSD17B2*, as there was no indication that they would be related to function. However, because of the interesting result in *IRS1*, we decided to genotype additional SNPs in genes in the IGF pathway, including two known functional SNPs, one in *PI3K* and one in *SHC1*, both active components of the IGF signaling pathway acting through the IGF1 receptor, and to examine genetic variation in *IRS1, IGFBP1, IGFBP3, SHBG, IGF1*, and *IGF1R* through looking at haplotypes by performing genotyping using haplotype-tagging SNPs. Thus, we have expanded the set of genes and methodology for investigating the association of genetic variation in these genes and prostate cancer risk.

Task 6: Read genotypes and enter into our Sybase database.

Genotypes that have been completed are currently entered in an excel spreadsheets. All genotypes generated in Task 5 have been entered into spreadsheets for analysis in Aim 2.

Aim 2: To statistically analyze the association of genes assayed in Aim 1 with prostate cancer age at diagnosis and aggressiveness, as measured by Gleason score and tumor stage-related variables. Aim 3: To statistically analyze the association of genes assayed from Aim 1 with occurrence of prostate cancer.

Task 7: Design data entry forms for entering data into Sybase. COMPLETED PREVIOUSLY, BUT NOT USING.

This task was completed so that we can download the data into Sybase. However, since it is a finite amount of data, it is better to use Excel spreadsheets that are uploaded for analysis with the statistical package SAS. Therefore, we are not using Sybase for storing the data.

Task 8: Edit data. Add data from medical records and Utah Cancer Registry. COMPLETED PREVIOUSLY.

The prostate cancer cases were diagnosed from 1992-2000. Age at diagnosis ranged from 45-78 years with a mean age of 62.6 years and a median age of 63 years. Of the tumors, 10 were well-differentiated, 139 were moderately differentiated and 50 were poorly differentiated. Thirteen of the cases had another type of cancer, either previous to or after diagnosis of prostate cancer. We obtained follow-up data on these cases with the dates of last follow-up ranging from 2000-2002. These data are in the excel spreadsheet with the genotypes. Of the 199 prostate cancer cases, 15 are deceased including 1 case diagnosed at 49 years of age who died from metastatic prostate cancer.

Task 9: Months 25-27: Test models and analysis methodologies. COMPLETED PREVIOUSLY. However, in this past year, we determined which SNPs were needed to tag the haplotypes for *IGF1* and *IGF1R*.

Gleason scores are being placed into groupings commonly used in clinical prognosis. Group 1 is Gleason 1-3 (none in this study); group 2 is Gleason 4-6; group 3 is Gleason 7; and group 4 is Gleason 8-10. Unconditional logistic regression models are being used to assess the main effects of the genetic variants on occurrence of prostate cancer. Gene x gene interactions are analyzed by logistic regression using the Wald $\chi 2$ test to determine significant differences in slopes. Logistic regression for a polychotomous outcome is being used to assess associations with Gleason score (≤ 6, 7, and ≥ 8). Gene x gene interactions for Gleason score are not being analyzed as there are too few individuals with Gleason scores 8-10 in order to reliably fit a model with interactions. Since the majority of the population was non-Hispanic white, adjustment for racial group is not being performed.

As mentioned above, we are using a haplotype-tagging approach to examine the genetic variation in *SHBG, IRS1, IGFBP1, IGFBP3, IGF1* and *IGF1R*. This allows us to examine the genetic variation across the entire gene in order to not miss a possible association within the gene. There are additional steps to haplotype analysis that are not present in traditional, genotype-based case-control studies. First, a set of SNPs must be selected that will mark the common haplotypes in the population. These SNPs are commonly referred to as haplotype-tagging SNPs. The next step is the assignment of haplotypes to the case and control individuals, based on their haplotype-tagging SNP genotypes. Without genotype information in the parents or a direct molecular assay of individual chromosomes, the haplotypes must be assigned based on a probability model. We have developed algorithms for selecting haplotype-

tagging SNPs and estimating haplotype assignments for the sampled individuals. The second algorithm assigns haplotypes to each individual, based on the individuals' genotype data and the estimated population haplotype frequencies. The output is a matrix with a column for each of the common haplotypes present in the study population (frequency greater than 0.05) and a row for each individual. A logistic regression can be carried out with the haplotype data to estimate the risk of disease associated with each haplotype.

Table 1 shows the number of SNPs identified and then the number of SNPs needed to tag the haplotype for each of the genes.

Table 1. Total number of SNPs, number of SNPs with > 5% frequency, and number of haplotype-tagging SNPs (ht-SNPs)

Gene	Total # SNPs	# SNPs >5% frequency	# ht-SNPs
IGF1	133	37	8
IGF1R	372	139	18
IGFBP1	63	17	3
IGFBP3	52	25	4
IRS1	28	10	4
SHBG	17	7	4
Total			41

Task 10: Months 26-36: Perform statistical analyses as outlined in Methods.

Completed in past years for *CYP17, IGF-1 str, CYP11A, VDR-BsmI, VDR-Taq1, VDR polyA, IRS1, SHBG,* and *SRD5A2-str*, the *INS, IGF1,* and *IRS2* variants with risk of prostate cancer and Gleason scores. Haplotype analysis also was previously reported for *SHBG* and *IGFBP1.*

No statistical analyses of the association analysis were performed during this period. We are waiting to complete all the genotyping. We will then also explore gene x gene interactions for genes within the same pathway.

Task 11: Months 34-36: Prepare and submit final report and manuscripts.
Task 11 has not been started. A no-cost extension has been granted so that we can perform the additional genotyping.

KEY RESEARCH ACCOMPLISHMENTS:

- Genotyping and statistical analyses of 14 genes and 31 variants completed in 199 cases and 254 controls. Statistical analyses of 10 genes and 18 variants completed in 199 cases and 254 controls.
- Published a manuscript on our positive finding of *IRS1.*
- Identified and designed assays for haplotype-tagging SNPs in *IGFBP1, SHBG, IGF1R, IRS1, IGF1,* and *IGFBP3.*

REPORTABLE OUTCOMES: Our manuscript on our positive finding of *IRS1* was accepted and published. It is included in the appendix.

CONCLUSIONS: The *IRS1* G972R GR/RR genotypes are associated with a 2.7-fold increased risk of prostate cancer risk and the *IRS2* G1057D GD/DD genotypes are significantly associated with cancer aggressiveness as measured by Gleason score. These results provide additional support for an insulin-like growth factor and/or insulin pathway in the etiology of prostate cancer, and suggest that there are common variants associated with increases in prostate cancer risk and cancer aggressiveness. Validation studies need to be performed to confirm these findings.

REFERENCES: None

APPENDICES: Manuscript entitled Prostate cancer risk and *IRS1, IRS2, IGF1*, and *INS* polymorphisms: strong association of *IRS1* G972R variant and cancer risk.

The Prostate 64:168–174 (2005)

Prostate Cancer Risk and *IRS1*, *IRS2*, *IGF1*, and *INS* Polymorphisms: Strong Association of *IRS1* G972R Variant and Cancer Risk

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BACKGROUND. As cellular proliferation is central to the carcinogenic process, pathways that regulate proliferation may be important. Therefore, genes in the insulin and the insulin-like growth factor signaling pathways are plausible candidates for susceptibility genes for prostate cancer. We hypothesized that functional polymorphisms in *INS*, *IRS1*, *IRS2*, and *IGF1* may be associated with prostate cancer.

METHODS. We studied 199 incident prostate cancer cases and 267 age-matched controls. Genotyping was performed for the *INS* +1127 Ins-PstI, *IRS1* G972R, *IRS2* G1079D, and the *IGF1* CA-repeat polymorphisms. Outcomes were prostate cancer, Gleason score, and AJCC stage.

RESULTS. The *IRS1* G972R GR/RR genotypes were associated with a significant 2.8-fold increased risk for prostate cancer (95% CI 1.5–5.1, $P = 0.0007$). The other variants were not significantly associated with prostate cancer. The *IRS1* G972R GR/RR genotypes were also significantly associated with more advanced Gleason score ($P = 0.001$) and AJCC stage ($P = 0.004$).

CONCLUSIONS. These results support a role of the insulin and/or insulin-like growth factor pathways in the etiology of prostate cancer. *Prostate 64: 168–174, 2005.* © 2005 Wiley-Liss, Inc.

KEY WORDS: **prostate cancer; insulin receptor substrate 1; IRS1; IRS2; INS; IGF1**

INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer in men (230,110 estimated new cases to be diagnosed in 2004) and the second most common cause of cancer mortality (an estimated 29,900 deaths) in the US [1]. One in six men will be diagnosed with prostate cancer over the course of a lifetime. Prostate cancer is likely caused from complex interactions among genetic, endocrine, and environmental factors. Ethnic differences in risk suggest that in addition to environmental factors, common genetic variants with low penetrance and high population attributable risk may play an important role in the etiology of prostate cancer.

As cellular proliferation is central to the carcinogenic process, pathways that regulate proliferation may play an important role. The insulin-like growth factor signaling pathway is one such pathway. Elevated plasma levels of insulin-like growth factor 1 (IGF-1) [2–4] and a high IGF-I:IGFBP-3 (insulin-like growth factor binding protein 3) ratio are associated with an increased risk of prostate cancer [2,5,6] and are

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predictors of advanced-stage prostate cancer [7]. Variation in serum IGF-1 levels has been associated with a CA-repeat polymorphism 1 kb upstream of the IGF1 transcription start site [8]. IGF-1 is the primary ligand for the insulin-like growth factor receptor 1 (IGF-1R), which regulates apoptosis and cell proliferation through activation of downstream pathways [9,10]. Insulin receptor substrate 1 (IRS-1) is the primary docking molecule for the receptor and is required for activation of the phosphoinositol-3-kinase (PI3K) pathway, which regulates IGF-mediated survival, enhancement of cellular motility, and anti-apoptosis; and for activation of the RAS-MAPK pathway, which regulates cell proliferation [11,12]. The Gly972Arg polymorphism in *IRS1* has been reported to impair insulin-stimulated signaling [13]. IRS-2 also acts as a docking molecule and is involved in insulin signaling [14]. An *IRS2* Gly1057Asp polymorphism has been associated with insulin sensitivity and may subtly mediate interaction with downstream signaling molecules [15].

Insulin is a potent mitogen in prostate cells. It indirectly affects androgen levels through regulation of sex hormone binding globulin (SHBG) levels. Insulin directly regulates insulin-like growth factor binding proteins (IGFBPs) levels and therefore affects bioavailable IGF-1 [16,17]. In a case-control study in China, men with fasting serum insulin levels in the highest tertile had a 2.5-fold increased risk of prostate cancer [18]. A recent study reported an association of an insulin (INS) gene polymorphism (+1127 INS-PstI) and prostate cancer in African-American and Caucasian diabetics [odds ratio (OR) 3.14; $P = 0.0008$] [19].

In this study, we examined the association of the *IGF1* CA-repeat, the *IRS1* G972R, the *IRS2* G1057D, and the *INS* +1127 INS-PstI polymorphisms and prostate cancer risk in a case-control study.

MATERIALS AND METHODS

Subjects

Prostate cancer cases. Newly diagnosed cases of prostate cancer (n = 199) were enrolled at the time of radical prostatectomy between 1992 and 1999 at the University of Utah Medical Center. All cases were consented per an Institutional Review Board approved protocol. Information on family history of prostate cancer in first-degree relatives was obtained. All men were pathological T1-T4, No, Mo at the time of surgery. Ninety-seven percent of cases were non-Hispanic Caucasian. Gleason score, AJCC stage, capsular extension status, surgical margin status, and seminal vesicle involvement data were obtained from medical records or from the Utah Cancer Registry. Of the tumors, 10 were well-differentiated, 139 were moderately differentiated, and 50 were poorly differentiated.

Controls. Male controls were selected that were age-matched (within 5 years of birth year) with no previous history of prostate cancer. Ninety-eight percent of controls were non-Hispanic Caucasian. All controls were consented per an Institutional Review Board approved protocol. These controls were selected from within a Utah population-based set of controls previously identified from driver's license lists if younger than 65 years and Health Care Financing Administration (HCFA) for those 65 years of age and older. Data were available on prostate or other cancer diagnoses in these men.

Genotyping

DNA for genotyping was obtained from peripheral blood collected following prostatectomy (for patient specimens) and at the time of enrollment (for controls) using routine procedures. Genotyping was performed for the *IGF1* CA repeat, the *IRS1* 972 Gly > Arg (G972R), the *IRS2* 1057 Gly > Arg variant (G1057D), and the *INS* +1127 Ins-PstI variants. Genotypes were available for 193 cases and 263 controls for *IGF1*, 180 cases and 255 controls for *INS*, 191 cases and 260 controls for *IRS1*, and 177 cases and 256 controls for *IRS2*.

IRS1. The G972R polymorphism was detected using PCR amplification with primers 5′-CTTCTGTCAGG-TGTCCATCC (forward) and 5′-TGGCGAGGTGTC-CACGTAGC (reverse) [20]. PCR cycling consisted of an initial denaturation at 94°C for 2 min, 10 cycles at 94°C 10 sec, 60°C 10 sec, and 72°C 10 sec followed by 30 cycles at 94°C 10 sec, 55°C 10 sec, and 72°C 10 sec. *BstN*I was used to digest the PCR products following manufacturer's instructions. Alleles were scored as either G for glycine or R for arginine (absence or presence of the restriction site, respectively).

IRS2. The G1057D polymorphism was detected using PCR amplification with primers were 5′-ACTCCCGA-CACCTGCTCCAT(forward) and 5′-CCCGTGGGCTC-CTTGGAC (reverse). PCR reactions were conducted in 12.5 μl volumes, containing 20 ng genomic DNA, 100 μM dNTP, 0.4 μM each primer, 1× PCR buffer (Qiagen), 1× Q solution (Qiagen) and 0.3 units Taq DNA polymerase (Qiagen). PCR cycling consisted of an initial denaturation at 2 min followed by 35 cycles of 94°C for 15 sec, 62°C for 15 sec, and 72°C for 30 sec. *Ban*I was used to digest the PCR products following manufacturer's instructions. Alleles were scored as either G (178 bp and 181 bp products) or D (359 bp

product) (presence or absence of the restriction site, respectively).

INS. The INS +1127 C/T polymorphism, located in the 3′ UTR, 9 bp downstream of the stop codon was amplified using PCR primers: 5′-AGAAGCGTGG-CATTGTGGAA (forward) and 5′-AGCAGGCAGC-CAACAGGCA (reverse). PCR conditions consisted of a 2 min denaturation at 94°C, followed by 35 cycles of 94°C for 15 sec, 60°C for 15 sec, and 72°C for 30 sec. *Pst*I was used to digest the PCR products, following manufacturer's instructions. Alleles were scored as either C (322 bp fragment) or T (236 bp and 86 bp) (absence or presence of the restriction site, respectively).

IGF1. The IGF1 CA repeat was amplified using PCR primers 5′-GCTAGCCAGCTG GTGTTATT (forward) and 5′-ACCACTCTGGGAGAAGGGTA (reverse) [8]. PCR conditions consisted of a 2-min denaturation at 94°C followed by 30 cycles of 94°C 10 sec, 57°C 10 sec, and 72°C 15 sec. Alleles were assigned by size of fragment in base pairs and classified as "192" or not "192." "192" is the PCR product size of the most common allele, which contains 19 CA repeats.

Statistical Analysis

SAS statistical package, version 8.2, was used to conduct the analyses. Analyses included evaluating the distribution of genotypes in the population, the associations of individual variants with prostate cancer risk, Gleason score, and AJCC stage, as well as the joint effect of variants on prostate cancer risk. Hardy–Weinberg equilibrium and allele frequency were determined using the SAS Genetics program. A case-control design was used for assessing outcomes. Dominant models (having at least one variant allele, which therefore included heterozygotes and homozygote variant genotypes) were used as there were too few homozygous variant individuals for *IRS1* and *INS* in order to have sufficient power to evaluate co-dominant or recessive models. Gleason scores were placed into groupings commonly used in clinical prognosis. Group 1 was Gleason 1–3 (none in this study); Group 2 was Gleason 4–6; Group 3 was Gleason 7; and Group 4 was Gleason 8–10. Unconditional logistic regression models were used to assess the main effects of the genetic variants on occurrence of prostate cancer. Gene × gene interactions were analyzed by logistic regression using the Wald χ^2 test to determine significant differences in slopes. Logistic regression for a polychotomous outcome was used to assess associations with Gleason score ($\leq$6, 7, and $\geq$8) and AJCC stage. In these analyses, each Gleason score and AJCC stage were compared to controls for a case-control comparison. In a case–case comparison, Gleason scores 7 and 8–10 were compared to Gleason scores 4–6 and AJCC stages 2–4 were compared to AJCC stage 1. Age was included as a covariate in all analyses. Since the majority of the population was non-Hispanic Caucasian, adjustment for racial/ethnic group was not performed. Gene × gene interactions for Gleason score and AJCC stage were not analyzed as there were too few individuals with Gleason scores 8–10 in order to reliably fit a model with interactions.

RESULTS

The cancer characteristics for the cases and the genotype frequencies of the cases and controls are provided in Table I. All variants were in Hardy–Weinberg equilibrium (HWE) (Table I). Age at diagnosis ranged from 45 to 78 years with a mean age of 63 years. The majority of cases were diagnosed under age 70 years. The age for controls at enrollment ranged from 40 to 79 years with a mean age of 64 years.

For the association of the genetic polymorphisms with risk of prostate cancer, odds ratios (OR) and their corresponding 95% confidence intervals (CI) are presented in Table II. There was a significant association of *IRS1* 972 GR/RR genotypes with prostate cancer risk ($P = 0.0007$) with an OR of 2.8 (95%CI = 1.5–5.1) The other three variants were not significantly associated with prostate cancer risk. There were no significant associations of gene × gene interactions and cancer risk (data not shown).

For Gleason score, there was a significant association of *IRS1* 972 GR/RR genotypes with Gleason category ($P = 0.001$) (Table III), with a 6.3-fold increased risk of Gleason scores 8–10 (95% CI 2.3–17.6). We did not detect significant associations with the other three variants and Gleason category (Table III), nor for any variants in the case–case comparison (data not shown). Similarly, there was a significant association of *IRS1* GR/RR genotypes with AJCC stage ($P = 0.004$), in that those with the greatest risk had more advanced AJCC stage. The other variants did not appear to be significantly associated with AJCC stage (Table IV). *IRS2* GD/DD genotypes appeared to be protective for stage 1 prostate cancer, but not for later stages.

DISCUSSION

The IGF and insulin pathways may play important roles in risk and progression of prostate cancer. Therefore, we evaluated genetic variants in four genes in these pathways. We present evidence that the *IRS1* G972R variant (GR/RR genotypes) is significantly associated with prostate cancer, conferring a 2.8-fold increased risk. We were unable to confirm the

TABLE I. Description of the Study Population

	Cases (%)	Controls (%)
Total available	199	267
Mean age (and age range) at diagnosis for cases/enrollment for controls	63 years (45–78 years)	64 years (40–79)
Gleason category		NA
1 (Gleason 1–3)	0 (0)	
2 (Gleason 4–6)	103 (51.7)	
3 (Gleason 7)	71 (35.7)	
4 (Gleason 8–10)	25 (12.6)	
Stage		NA
T1	26 (13.1)	
T2	100 (50.3)	
T3	56 (28.1)	
T4	15 (7.5)	
IGF1	193	263
192/192	78 (40.4)	107 (40.7)
192/−192 alleles	86 (44.6)	124 (47.1)
No 192 bp allele	29 (15.0)	32 (12.2)
Allele frequency (HWE *P*-value)	0.37 (0.81)	0.36 (0.91)
INS	180	255
+1127 C/C	118 (65.6)	160 (62.7)
+1127 C/T	50 (27.8)	81 (31.8)
+1127 T/T	12 (6.7)	14 (5.5)
Allele frequency (HWE *P*-value)	0.21 (0.16)	0.21 (0.69)
IRS1	191	260
Gly/Gly	156 (81.7)	241 (92.7)
Gly/Arg	30 (15.7)	17 (6.5)
Arg/Arg	5 (2.6)	2 (0.8)
Allele frequency. (HWE *P*-value)	0.10 (0.14)	0.04 (0.17)
IRS2	177	256
Gly/Gly	85 (48.0)	119 (46.5)
Gly/Asp	67 (37.9)	106 (41.4)
Asp/Asp	25 (14.1)	31 (12.1)
Allele frequency (HWE *P*-value)	0.33 (0.16)	0.33 (0.62)

TABLE II. Association of Genetic Variants With Prostate Cancer Occurrence

Gene	Number of Cases	Number of Controls	OR (95% CI)[a]	*P*-value
IGF1				
192/192	78	107	1.0	
Non-192/192 No 192 allele	115	156	1.0 (1.0–1.1)	0.95
INS				
CC	118	160	1.0	
CT/TT	62	95	0.9 (0.6–1.3)	0.59
IRS1				
GG	156	241	1.0	
GR/RR	35	19	2.8 (1.5–5.1)	0.0007
IRS2				
GG	85	119	1.0	
GD/DD	92	137	1.0 (0.7–1.4)	0.87

[a]Age was a covariate in all analyses.

TABLE III. Association of Genetic Variants With Gleason Category

	OR (95% CI)[a] [n cases]			χ^2 *P*-value across Gleason categories
	Gleason categories			
Gene	4–6	7	8–10	
IGF1				
192/192	1.0 [46]	1.0 [20]	1.0 [12]	
Non-192/192 No 192 allele	0.8 (0.5–1.3) [56]	1.6 (0.9–2.9) [48]	0.6 (0.3–1.5) [11]	0.13
INS				
CC	1.0 [65]	1.0 [41]	1.0 [12]	
CT/TT	0.8 (0.5–1.3) [31]	0.9 (0.5–1.6) [22]	1.3 (0.5–3.1) [9]	0.40
IRS1				
GG	1.0 [85]	1.0 [57]	1.0 [14]	
GR/RR	2.5 (1.3–5.1) [15]	2.4 (1.1–5.4) [11]	6.3 (2.3–17.6) [7]	0.001
IRS2				
GG	1.0 [49]	1.0 [30]	1.0 [6]	
GD/DD	0.9 (0.5–1.4) [49]	0.8 (0.5–1.5) [29]	2.0 (0.8–5.4) [14]	0.75

[a]Odds Ratios and 95% CI determined by polychotomous regression comparing risk at each Gleason group to controls.

previously reported [19] association of the *INS* variant. We also found the most significant associations between the *IRS1* 972R variant and more advanced Gleason score and AJCC stage, measures of possible cancer aggressiveness.

The IRS protein family contains several members, of which IRS-1 and IRS-2 are expressed in almost all cells and tissues [21–23]. IRS-1 and IRS-2 could play roles in either or both IGF and insulin pathway signaling, as they are critical adaptor proteins for both. IRS-1 is the primary docking protein of the IGF-1R, which mediates cell growth, adhesion, transformation, and protection from apoptosis [24,25]. IGF-1R expression has been shown to be up-regulated in primary prostate cancer as compared to benign prostatic hyperplasia [26,27]. Down-regulation of IGF-1R leads to apoptosis of cancer cells [25]. In a study where IGF-IR gene expression was reduced in stably transfected PC-3 cells, there was a significant reduction of PC-3 cell invasion and proliferation in vitro, as well as an increased rate of spontaneous apoptosis [28]. Thus, up- or down-regulation of IGF-1R activation could have significant impact on prostate cancer development and progression.

IRS-1 is a critical docking protein for downstream signaling of IGF-1R through the PI3K pathway, and IRS-1 or SHC are needed for activation of the RAS-ERK pathway. In experiments with LNCaP cells (human

TABLE IV. Association of Genetic Variants With AJCC Stage

	OR (95% CI)[a] [n cases]				χ^2 *P*-value across stage
	Stage				
Gene	1	2	3	4	
IGF1					
192/192	1.0 [9]	1.0 [42]	1.0 [22]	1.0 [4]	
192/no 192 No 192 allele	1.3 (0.6–3.0) [17]	0.9 (0.6–1.4) [55]	1.0 (0.6–1.8) [32]	1.7 (0.5–5.6) [10]	0.83
INS					
CC	1.0 [21]	1.0 [59]	1.0 [28]	1.0 [12]	
CT/TT	0.2 (0.1–0.8) [3]	0.9 (0.6–1.5) [32]	1.3 (0.7–2.3) [21]	0.9 (0.3–2.9) [5]	0.19
IRS1					
GG	1.0 [22]	1.0 [76]	1.0 [46]	1.0 [10]	
GR/RR	2.3 (0.7–7.4) [4]	3.3 (1.7–6.6) [20]	1.9 (0.8–4.9) [7]	5.0 (1.5–17.7) [4]	0.004
IRS2					
GG	1.0 [14]	1.0 [47]	1.0 [18]	1.0 [5]	
GD/DD	0.7 (0.3–1.6) [11]	0.8 (0.5–1.3) [45]	1.4 (0.7–2.6) [28]	1.2 (0.4–3.9) [7]	0.61

[a]Odds ratio and 95% confidence intervals were determined by polychotomous regression comparing each disease stage to control population.

prostatic cell lines that do not express IRS-1, have low levels of IGF-IR and have a frame-shift in PTEN), ectopic expression of IRS-1 resulted in an increase in PI3K, resulting in increased cell adhesion and decreased cell motility by an IGF-1 independent mechanism [29]. In these LNCaP cells, IRS-1 is serine phosphorylated by PI3K and interacts with integrins [30]. The *IRS1* G972R polymorphism has been shown to have an effect on insulin-stimulated signaling. Almind et al. [13], using a cultured myeloid progenitor cell and Hribal et al. [31], studying L6 skeletal muscle cells, showed that the 972R variant decreased insulin-stimulated signaling by 25%, largely through the PI3K pathway due to decreased binding of the PI3K p85 subunit to IRS1. In MCF-7 breast cells, IRS-1 degradation is blocked by PI3K inhibitors, suggesting a direct negative-feedback mechanism of PI3K that degrades IRS-1 and thus blocks further IGF signaling [32]. Therefore, one possible hypothesis for the increased risk of prostate cancer with the *IRS1* G972R variant is that with decreased PI3K binding, less IRS-1 is degraded resulting in longer signaling through both the P13K and RAS-ERK pathways and thus increased risk of prostate cancer. Secondly, IRS-1 is a known regulator of cell size and has been shown to account for approximately 50% of cell size in mice, flies, and cells in culture, and to play a role in transformation [discussed in [9]]. Thus, this variant may be affecting binding to upstream binding factor (UBF), critical to cell growth. IRS-1 is also known to interact with integrins to promote cell adhesion [33]. Therefore, a possible hypothesis for the association with AJCC stage is that this variant affects interaction with integrins resulting in lower adhesion and therefore increased invasion.

A strength of this study is the investigation of several variants in several genes along a pathway that may be associated with prostate cancer. Focusing on a disease pathway, rather than isolated genetic polymorphisms allows for a more complete examination of a pathway and provides more information on the importance of the pathway to the etiology of the disease. A limitation of this study is that we examined only four polymorphisms in four genes along this pathway. There may be other polymorphisms in these and other genes in this pathway that may be involved in the prostate cancer. A second limitation is that this is a small case-control study such that there was limited power for evaluating Gleason score and AJCC stage and detection of gene × gene interactions. It is important to evaluate the statistical significance of the results in the context of the strengths and limitations of a study. Two recent publications discuss the assessment of positive results from association studies [34,35], considering the prior probability of a true association, the observed *P*-value and the power of the study. The probability that the association between the *IRS1* G972R variant and prostate cancer is a true association is strengthened by the strong prior probability that IRS–1 is related to cancer risk and the low *P*-value, while it is tempered by the limitation of the modest sample size. These results need to be verified in a larger prostate cancer study.

It is important to examine this pathway, and, in particular, the *IRS1* G972R variant in other cancers. Recently, we observed an association of this variant with increased colon cancer risk (OR 1.4; 95% CI 1.1–1.9), but not with rectal cancer [36] in a large case-control study of colon and rectal cancers. For colon cancer, we also observed that those individuals with the *IRS1* R variant allele and an *IGF1* non-192 bp variant and with the *IRS1* R variant allele and an *IGFBP3*-202 C variant allele were at 2-fold and 1.7-fold increased risk of colon cancer, respectively. This suggests that IRS-1 may play a role in a range of cancers. Studies of breast cancer are underway.

CONCLUSIONS

The *IRS1* G972R GR/RR genotypes are associated with a 2.8-fold increased risk of prostate cancer risk and are significantly associated with cancer aggressiveness as measured by Gleason score and AJCC stage. These results provide additional support for an insulin-like growth factor and/or insulin pathway in the etiology of prostate cancer. In future studies, additional variants and genes in the IGF pathway should be explored in order to further our understanding of the role of this pathway and prostate cancer. It is hoped that as IGF-targeting therapies for prostate cancer are being investigated, these results can provide additional clues for design of approaches.

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